American Fern Journal 85(4):341–360 (1995)

Phylogeny and Generic Circumscriptions of Cheilanthoid Ferns (Pteridaceae: Cheilanthoideae) Inferred from *rbcL* Nucleotide Sequences

GERALD J. GASTONY and DAVID R. ROLLO
Department of Biology, Indiana Univeristy, Bloomington, IN 47405–6801

ABSTRACT.—Nucleotide sequences of the chloroplast DNA gene *rbcL* were determined for 25 species of cheilanthoid ferns (Pteridaceae subfamily Cheilanthoideae). Together with GenBank sequences of an additional cheilanthoid and a 14-species outgroup comprising Pteridaceae, Vittariaceae, and *Coniogramme japonica,* these were analyzed cladistically by maximum parsimony to gain insights into cheilanthoid phylogeny and generic circumscriptions, which have long stymied pteridologists. Our analysis yielded 4 equally most parsimonious trees of 1570 steps. Two of these trees varied only in the relative positions of *Onychium* and *Pteris* in the global outgroup. The other two varied only in the relative positions of *Pellaea pringlei* and *P. rotundifolia* in the ingroup. Results based on *rbcL* sequences are concordant with those of recent studies of *Argyrochosma, Hemionitis,* and *Bommeria* that utilized a broad suite of characters, suggesting that *rbcL*-based inferences in less meticulously studied cheilanthoid groups also convey meaningful information. Among the insights into cheilanthoid phylogeny and generic circumscriptions offered by *rbcL* sequences are the following. *Llavea* does not belong in subfamily Cheilanthoideae. *Pellaea* and *Cheilanthes* are polyphyletic. The recent removal of 21 species from *Notholaena* to *Cheilanthes* and the segregation of *Argyrochosma* are supported. The transfer of *Hemionitis elegans* to *Bommeria* is strongly supported, but the removal of the group of *Doryopteris concolor* to *Cheilanthes* is not. *Trachypteris* is sister to *Doryopteris.* The segregation of some small genera from *Cheilanthes* is supported, but these require further study through inclusion of additional taxa. *Bommeria* is the most basal ingroup element in this analysis.

The cheilanthoid homosporous ferns (Pteridaceae subfamily Cheilanthoideae) are a virtually worldwide group with many or most species adapted to seasonally xeric habitats, as in the southwestern United States and Mexico. According to their most recent comprehensive treatment (Tryon et al., 1990), cheilanthoids comprise over 300 species in 12 genera: *Cheilanthes* (150), *Notholaena* (40), *Pellaea* (35), *Doryopteris* (25), *Coniogramme* (20), *Adiantopsis* (7), *Hemionitis* (7), *Paraceterach* (7), *Bommeria* (4), *Trachypteris* (3), *Cryptogramma* (2), and *Llavea* (1). Tryon et al.'s rather conservative taxonomy did not recognize a number of genera that others segregate from *Cheilanthes,* for example, *Mildella* (Copeland, 1947; Hall and Lellinger, 1967) *Sinopteris* (Christensen and Ching, 1933), *Aleuritopteris* (Ching, 1941; Saiki, 1984), *Cheiloplecton* (Copeland, 1947; Smith, 1981; Mickel and Beitel, 1988), and *Aspidotis* (Copeland, 1947; Lellinger, 1968, 1985; Smith, 1975). Nor did Tryon et al. (1990) accept *Argyrochosma,* recently segregated from *Notholaena* (Windham, 1987).

Taxonomic problems in cheilanthoids are far deeper than conflicts over which segregate genera to recognize. Copeland (1947, p. 81) concluded that the cheilanthoid group, ". . . is, and has long been one of the most puzzling among all those of ferns." Circumscriptions of the four largest genera are wide-

ly recognized to rank among the most difficult problems in pteridology, and experts have often expressed frustration when trying to decipher natural evolutionary lineages that can be circumscribed as genera, subgenera, etc. Knobloch (1976) concluded that there is no certain way to separate *Cheilanthes* and *Notholaena.* If one arbitrarily attributes to *Notholaena* those species with a scarcely inturned marginal false indusium and to *Cheilanthes* those with this structure strongly inturned, one comes into conflict with the characters of *Pellaea,* which also has a false indusium. Smith (1981) observed that a satisfactory circumscription of these genera has not yet been attained, concluding that some species of *Notholaena* seem more closely related to species currently placed in *Cheilanthes* than to other species of *Notholaena* and that the circumscription of *Pellaea* is unsatisfactory. Mickel (1979) and Mickel and Beitel (1988) found meaningful generic circumscription so intractable that they combined all of *Notholaena* and *Cheilanthes* into a single enlarged genus *Cheilanthes,* while conceding that there are several distinct species groups in this complex (some recognized by others as segregate genera, based on character states with troublesome exceptions or intermediates, e.g., *Aleuritopteris, Aspidotis,* and *Sinopteris* noted above). Mickel (1974) further observed that the relationship of *Hemionitis* to other genera is not clear and called for further study and re-evaluation of a broad array of characters in *Hemionitis, Trachypteris, Bommeria,* and other genera. Stolze (1981) restated Copeland's (1947) assessment that *Cheilanthes* is a difficult and unsatisfactory genus and concluded that, "if anything, this is an understatement." Tryon (1956) noted that *Notholaena* could not be defined in a way that would set it apart from *Cheilanthes* and *Pellaea.* He observed that *Notholaena*'s circumscription might be resolved if *Cheilanthes* and *Pellaea* were well defined, but confessed that problems in those genera are as perplexing as those in *Notholaena.* Following many years of work with cheilanthoids, Tryon and Tryon (1973) concluded that, "There is an obvious need for the development of new data which will give a better insight into the evolutionary lines within the group," and noted that, "Convergence in adaptive morphology has undoubtedly been frequent among cheilanthoid ferns." Convinced that, ". . . convergence of single characters is especially common in this group," Tryon and Tryon (1982) transferred some species from *Notholaena, Pellaea,* and *Doryopteris* to *Cheilanthes* in an effort to clarify the taxonomy and relationships of those three genera, but this simultaneously made *Cheilanthes* (for which there is no modern revisionary study) a larger and admittedly more heterogeneous group. Unable to resolve *Cheilanthes* further with traditional taxonomic characters, they noted that, "Further study is needed to adequately characterize the evolutionary groups within it or to propose clearly merited generic segregates," and concluded that, "The cheilanthoid ferns have been the most contentious group of ferns with respect to a practical and natural generic classification." The most recent comprehensive taxonomic summary of cheilanthoid ferns (Tryon et al., 1990) concluded that, "Evolutionary lines within the subfamily and the relations of these to other groups in the Pteridaceae are scarcely known."

The main reason given for the taxonomic problems in this group is mor-

phological homoplasy associated with adaptation to xeric habitats, as expressed in quotations from Tryon and Tryon (1973, 1982) in the preceding paragraph. Haufler (1985) noted that leaves and other morphological features of xeromorphic ferns may be convergently modified through ecological adaptations, severely limiting their value in assessing evolutionary relationships, and Lellinger (1989) conceded that, "The taxonomic value of some traditionally generic characters is in doubt because of convergence due to harsh habitats." Morphology did serve as a useful component of Ranker's (1990) insightful analyses with a small group of *Hemionitis* and *Bommeria* species. Nevertheless, it is questionable whether solutions to many of the taxonomic difficulties across the breadth of the cheilanthoids would come from cladistic analyses using traditional morphological characters.

A potential solution to the longstanding problem with cheilanthoids is to use newly available molecular data at the level of DNA sequences, presumably unaffected by adaptation to arid habitats, to give new insight into the evolutionary lines within cheilanthoids. In particular, the nucleotide sequence of the *rbcL* gene encoded in the chloroplast genome seems appropriately divergent for use in phylogenetic reconstructions across the taxonomic diversity of cheilanthoid ferns. This gene, which encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, has been most useful in reconstructing phylogenies at diverse taxonomic levels in seed plants (Chase et al., 1993, and other papers in volume 80 number 3 of the Annals of the Missouri Botanical Garden). The present study provides cladistic analyses of *rbcL* sequences of a selected subset of species from several cheilanthoid genera to test the hypothesis that *rbcL* will provide the long sought, "New data which will give a better insight into the evolutionary lines within the group" (Tryon and Tryon, 1973).

Materials and Methods

This study provisionally follows the taxonomy of the most recent comprehensive treatment of cheilanthoid ferns, that of Tryon et al. (1990). Sporophytes collected directly in nature, or grown from spores taken from specimens provided by colleagues, were the source of DNAs used to generate the 25 new *rbcL* sequences in this study (Table 1). Total genomic DNAs were extracted from these plants by the methods of Yatskievych et al. (1988) or by those of Saghai-Maroof et al. (1984) as modified by Doyle and Doyle (1987), in the latter case with leaves ground directly in 65°C 2X CTAB (n-hexadecyl trimethylammonium bromide) solution or added to that solution after grinding to a powder in liquid nitrogen.

Polymerase chain reaction (PCR) amplifications using these DNA templates and primers 1F and 1351R (Table 2) produced a 1381 base pair (bp) fragment of the *rbcL* gene. Sequencing reactions used either genecleaned (Bio 101, La Jolla, CA) double-stranded PCR products or single-stranded DNA obtained via cloning PCR products, involving transfection with M13 helper phage (TA Cloning Kit and M13 from Invitrogen, San Diego, CA) and isolation and purification of single-stranded templates according to protocols in Sambrook et al. (1989).

TABLE 1. Sources of living material of ingroup species used to generate *rbcL* sequences for this study. Taxonomy follows that of Tryon et al. (1990). Voucher herbarium specimens are deposited at the Indiana University herbarium (IND), except for *Trachypteris pinnata,* which is at São José do Rio Preto (SJRP) in Brazil.

Species	Provenance	Collector(s)	GenBank Accession No.
Bommeria ehrenbergiana (Klotzsch) Underw.	Hidalgo, Mexico	*Yatskievych & Gastony 89-203*	U19497
Cheilanthes albofusca Baker	Yunnan, China	*Li & Xiang S-4L*	U19498
C. allosuroides Mett.	Jalisco, Mexico	*Yatskievych & Gastony 89-237*	U27239
C. aurea Baker	Oaxaca, Mexico	*Yatskievych & Gastony 89-256*	U28786
C. bonariensis (Willd.) Proctor	Michoacan, Mexico	*Yatskievych & Gastony 89-246*	U19499
C. decora (Brack.) R. & A. Tryon	Kaui, Hawaii	*Flynn s.n.*	U27446
C. duclouxii (Christ) Ching	Yunnan, China	*Li & Xiang S-18L*	U27447
C. horridula Maxon	Nuevo León, Mexico	*Gastony 90-10-1*	U27448
C. intramarginalis (Kaulf. ex Link) Hook. var. *serratifolia* (Hook. & Baker) C.C. Hall & Lell.	Hidalgo, Mexico	*Yatskievych & Gastony 89-207*	U27449
C. lanosa (Michx.) D.C. Eaton	Martin Co., Indiana	*Hegeman s.n.*	U27205
C. rigida (Sw.) Mett.	Puebla, Mexico	*Yatskievych & Gastony 89-284*	U29133
Doryopteris pedata (L.) Fée var. *palmata* (Willd.) Hicken	Hidalgo, Mexico	*Riba 1746*	U27206
Hemionitis elegans Davenp.	Oaxaca, Mexico	*Yatskievych & Gastony 89-258*	U27729
H. levyi Fourn.	Oaxaca, Mexico	*Yatskievych & Gastony 89-253*	U27725
Llavea cordifolia Lag.	Nuevo León, Mexico	*Yatskievych & Gastony 89-224A*	U27726
Notholaena delicatula Maxon & Weath.	Nuevo León, Mexico	*Yatskievych & Gastony 89-229*	U19500
N. fendleri Kunze	Sandoval Co., New Mexico	*Sullivan, Drummond, & Fitzpatrick s.n.*	U27727
N. rosei Maxon	Oaxaca, Mexico	*Yatskievych, Windham, & Ranker 83-453*	U27728
N. sulphurea (Cav.) J. Sm.	Puebla, Mexico	*Yatskievych & Gastony 89-248*	U28254
Pellaea andromedifolia (Kaulf.) Fée	Orange Co., California	*Gastony 86-8*	U19501
P. boivinii Hook.	Prov. Fianarantsoa, Madagascar	*Liede & Conrad 2626*	U29132
P. cordifolia (Sessé & Moc.) A.R. Smith	Jeff Davis Co., Texas	*Gastony 87-3*	U28253
P. pringlei Davenp.	Morelos, Mexico	*Gastony 87-11-16*	U28787
P. rotundifolia (G. Forst.) Hook.	Indiana University greenhouse specimen	*Gastony s.n.*	U28788
Trachypteris pinnata (Hook. f.) C. Chr.	Prov. Santa Cruz, Bolivia	*Windisch 6088*	U27450

TABLE 2. Location and base composition of amplification and sequencing primers used in this study. Positions in parentheses were synthesized with equal parts of the indicated bases.

Primer	5′ sequence 3′	Designed by
1F	ATG TCA CCA CAA ACA GA(G/A) ACT AAA GC	Mark Chase
220F	GTA GCT (G/A)CG GAA TCT TCT ACG GG	Gerald Gastony
440F	GGT AAT GTT TTT GGA TTT AAG GC	Gerald Gastony
660F	AAC GTG AAT TCC CAA CCG TTT ATG	Gerald Gastony
Z-895	GCA GTT ATT GAT AGA CAG AAA AAT CAT GGT	Gerard Zurawski
Z-1020	ACT TTA GGT TTT GTT GAT TTA TTG CGC GAT GAT T	Gerard Zurawski
Z-1204	TTT GGT GGA GGA ACT TTA GGA CAC CCT TGG GG	Gerard Zurawski
235R	GC GAC GGG CTC AAT ATC GTA GCA	Gerald Gastony
454R	TTC CAC CTG AAT ACC ATG GGG	Gerald Gastony
455R	ATA AGC AGG AGG AAT TCG (T/G)AG GTC TTC	Gerald Gastony
675R	CAA GTA ATG (C/T)CC CTT GAT TTC GCC CGT TTC	Gerald Gastony
Z-895R	ACC ATG ATT CTT CTG CCT ATC AAT AAC TGC	Gerard Zurawski
Z-1020R	ATC ATC GCG CAA TAA ATC AAC AAA ACC TAA AGT	Gerard Zurawski
1080R	ATC TTG GGT AAA ATA GAT GCC	Gerald Gastony
Z-1204R	CCC TAA GGG TGT CCT AAA GTT TCT CCA CC	Gerard Zurawski
1351R	C TTC ACA AGC AGC AGC TAG TTC AGG ACT CC	Mark Chase

Double-stranded sequencing involved standard Sanger dideoxy protocols (Hillis et al., 1990), with sequencing reactions containing 10% w/v acetamide to alleviate pausing or compressions in G-C rich regions. Templates were denatured for 3 minutes at 100°C, and primer annealing was carried out for 2 minutes on wet ice. Single-stranded sequencing followed the standard Sequenase version 2.0 protocol of the United States Biochemical Corporation. Sequences generated from cloned material were corroborated by comparison with regions sequenced by the double stranded method. Gels used Long Ranger acrylamide (FMC BioProducts, Rockland, ME) to provide approximately 250 base pairs of well-spaced bands. The 1F and 1351R primers and those provided by Gerard Zurawski (Zurawski and Clegg, 1993) were used to generate *rbcL* sequence for several cheilanthoid species. Based on these sequences, additional cheilanthoid-based primers (Table 2) were synthesized as sequencing progressed through the gene. M13 primers (Invitrogen, San Diego, CA) were also used in sequencing single-stranded cloned templates. With this suite of primers we were able to read 1325 bp of sequence between PCR primers. Because compressions at base positions 400–403 were unresolved in some ingroup taxa, these positions were excluded from our analyses, which were therefore based on 1321 nucleotides for each of the 25 ingroup species in Table 1. Sequences were read in a single direction except for occasional ambiguous regions that were resolved by reading both strands. The 25 new sequences generated for this analysis are deposited in GenBank, and their accession numbers are reported in Table 1. These nucleotide sequences plus that of *Cheilanthes* (*Doryopteris*) *concolor* supplied by Mitsuyasu Hasebe (GenBank U05621) served as the ingroup data matrix.

Results of the global *rbcL* analysis of Hasebe et al. (1994) guided the choice of outgroups for this analysis. That global analysis (relevant portion repro-

FIG. 1. Part of the global analysis consensus tree of Hasebe et al. (1994). Subfamily Cheilanthoideae of Pteridaceae is represented by the clade (*Doryopteris*, *Notholaena*, *Pellaea*). Bracketed taxa represent families Pteridaceae and Vittariaceae sensu Tryon et al. (1990). *Coniogramme* (placed in Pteridaceae subfamily Cheilanthoideae by Tryon et al., 1990) is sister to all other members of Pteridaceae plus Vittariaceae in Hasebe et al.'s (1994) *rbcL* analysis. It is therefore excluded from the cheilanthoid ingroup in this study and is instead used as part of the global outgroup. Two of the four equally most parsimonious trees in this study differ only in the relative positions of *Onychium* and *Pteris* in the outgroup. This ambivalence was also seen in the global analysis of Hasebe et al. (1994), as reflected in the polytomy in the clade (*Platyzoma* to *Pteris*).

duced as Fig. 1) indicated that cheilanthoids are sister to other members of the Pteridaceae plus the Vittariaceae sensu Tryon et al. (1990). Although Tryon et al. (1990) included *Coniogramme* in subfamily Cheilanthoideae, the global analysis placed *Coniogramme* well outside the cheilanthoid ferns and sister to all other Pteridaceae plus Vittariaceae sensu Tryon et al. (1990). In view of this, *Coniogramme* was excluded from the ingroup and was used instead as part of a global outgroup that comprises species 26–32 and 34–40 in Fig. 3. These 14 outgroup sequences were provided by Mitsuyasu Hasebe and Edmund Crane and are available in GenBank (see accession numbers in Appendix 1 of Hasebe et al. [1995]). The more extensive analyses (including several additional taxa) of Hasebe et al. (1995) were conducted concurrently with the writing of this cheilanthoid study. The maximum parsimony and maximum likelihood analyses of Hasebe et al. (1995; their Figs. 2–6) indicate that the *Adiantum* to *Vittaria* clade is itself directly sister to the cheilanthoids, unlike its position in Fig. 1, based on Hasebe et al. (1994). This refined positioning reinforces the appropriateness of the outgroup taxa employed herein.

The data matrix of 26 ingroup plus 14 outgroup sequences was cladistically analyzed by maximum parsimony using PAUP 3.1.1 (Swofford, 1993). The large size of this matrix necessitated a heuristic search. To minimize the possibility of PAUP's analysis being confined to a single class (island) of most parsimonious trees, (Maddison, 1991), a random addition sequence was specified with 1000 replicates, beginning with a randomly selected 10-digit starting

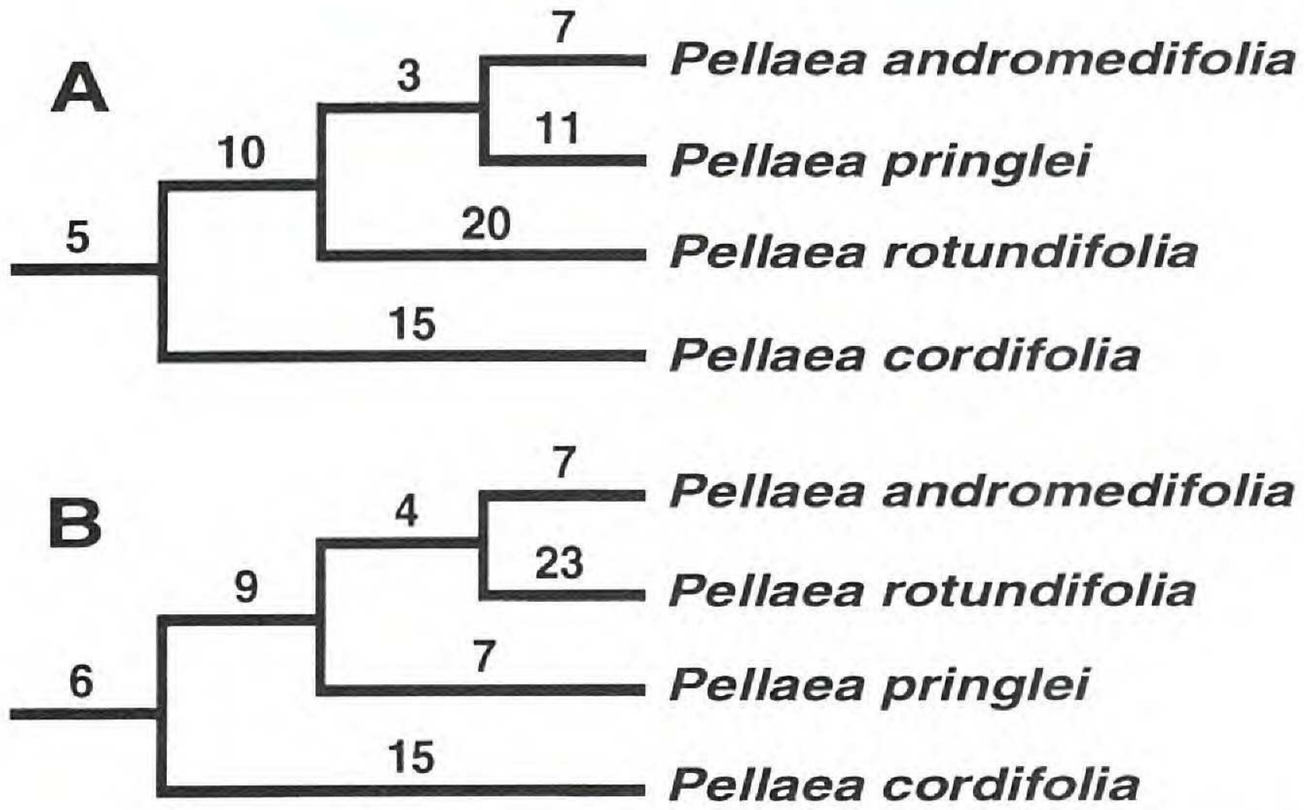

FIG. 2. Relative positioning of *Pellaea pringlei* and *P. rotundifolia* in A and B indicates sole variation in the two equally most parsimonious trees affecting ingroup topology in this study. A) *Pellaea rotundifolia* is sister to clade (*P. andromedifolia* + *P. pringlei*); B) *Pellaea pringlei* is sister to clade (*P. andromedifolia* + *P. rotundifolia*). The tree with the topology of A was arbitrarily chosen to represent the results of this study in Fig. 3.

seed and using TBR, MULPARS, and steepest descent (Swofford, 1993). Support for clades resolved by the foregoing basic analysis was determined by the bootstrap option of PAUP, using a random addition sequence for each of 1000 replicates, with TBR branch-swapping, MULPARS, and steepest descent suboptions in effect. The basic analysis required 11 hours and the bootstrap analysis 95 hours using a MacIntosh Quadra 800.

RESULTS

During the first random addition sequence replicate, PAUP found 4 equally most parsimonious trees of 1570 steps with a consistency index of 0.460, a retention index of 0.588, and a rescaled consistency index of 0.270. No new minimal length trees were found during any of the next 999 random addition sequence replicates, strongly suggesting that insularity (Maddison, 1991) is not a problem in this data set and that all of the most parsimonious trees have been found (Swofford, 1993, p. 35). Two of the four equally most parsimonious trees varied only by swapping the relative positions of *Onychium* and *Pteris* in the global outgroup. This ambivalence, also seen in the global analysis of Hasebe et al. (1994) as indicated by the polytomy in the consensus tree in Fig. 1, has no impact on the topology of the ingroup. The other two equally most parsimonious trees affect the ingroup only by switching the relative positions of *Pellaea pringlei* and *P. rotundifolia* as seen in Fig. 2A, B. All species in Fig. 2 are placed in *Pellaea* section *Pellaea,* except for *Pellaea rotundifolia,* which represents section *Platyloma* (Tryon and Tryon, 1982), but nests within *Pellaea* section *Pellaea* in Fig. 2. The long branch length leading to *P. rotundifolia* suggests that the ambivalence in positioning these two species may be resolved when an additional species of section *Platyloma* becomes available for analysis.

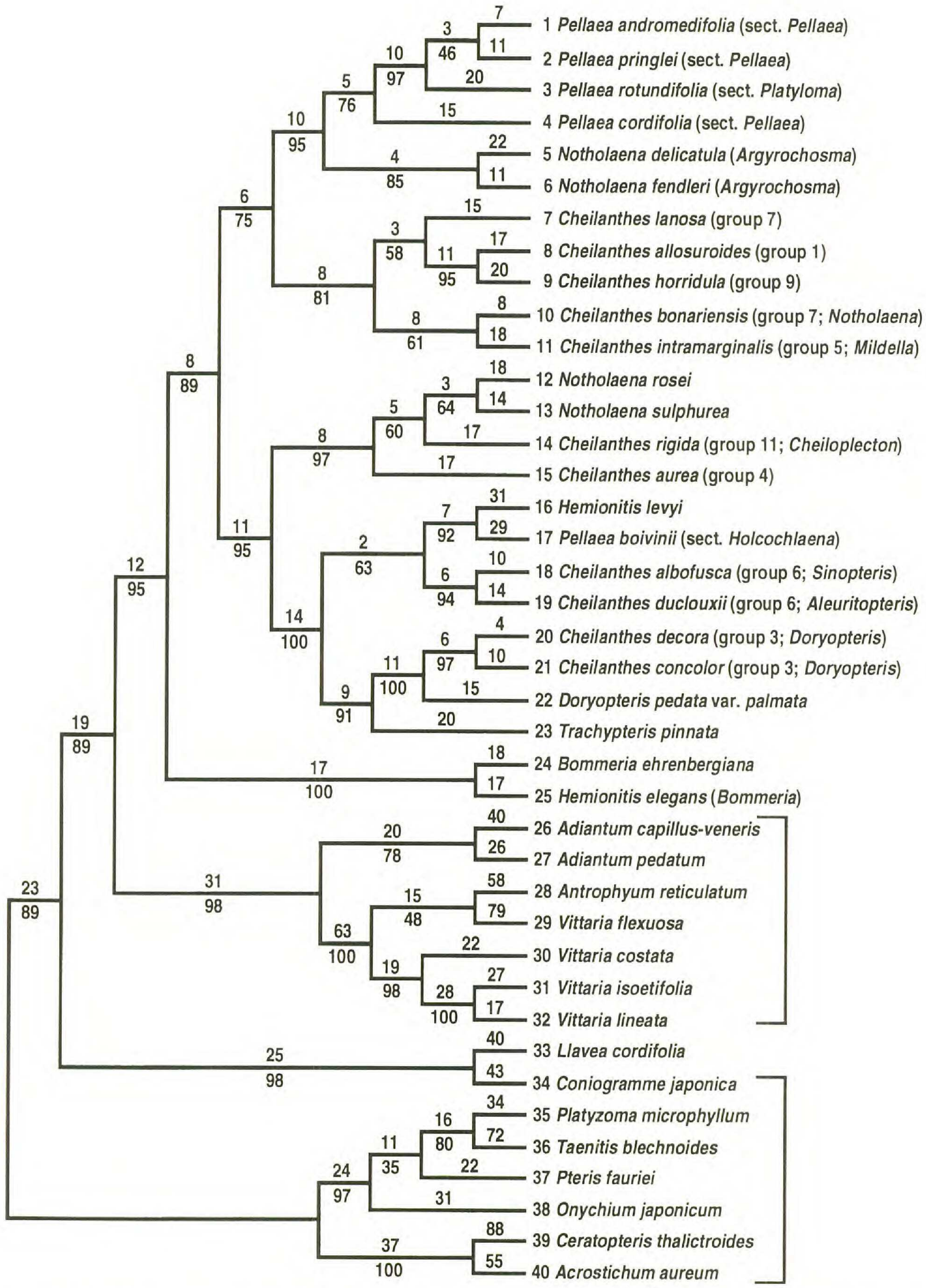

FIG. 3. One of two equally most parsimonious trees found for the ingroup in this study. The number of synapomorphies supporting each clade and autapomorphic branch lengths for terminal taxa are indicated above the lines. Numbers below lines represent bootstrap percentages based on 1,000 replicates. Species are numbered 1–40 to facilitate discussion in the text. Bracketed taxa

Of the two equally most parsimonious trees with regard to the ingroup, the one (Fig. 3) with the topology of Fig. 2A is arbitrarily chosen to represent the results of PAUP's analysis in the remainder of this paper. Except in cases where the rule of three synapomorphies (Felsenstein, 1985) is barely met, bootstrap support is quite high, in spite of the fact that the consistency index indicates fairly high homoplasy as is expected with large numbers of taxa and characters (Archie, 1989).

An unexpected result of this analysis is the position of *Llavea* (Fig. 3, species 33). PAUP was unable to root the most parsimonious trees such that the ingroup is monophyletic, because it could not place *Llavea* in the ingroup. Instead, *Llavea* is sister to *Coniogramme* (Fig. 3, species 34) in the outgroup in a clade strongly supported by 25 synapomorphies and a 98% bootstrap value.

At the top of the tree (Fig. 3), *Pellaea* sections *Pellaea* (represented by *P. andromedifolia, P. pringlei,* and *P. cordifolia*) and *Platyloma* (represented by *P. rotundifolia*) form a clade supported by 5 synapomorphies and a 76% bootstrap value, with section *Platyloma* surprisingly nested within section *Pellaea. Pellaea* section *Holcochlaena* (represented by *P. boivinii,* species 17), however, is deeply separated from its congeners, forming a fairly strongly supported clade with *Hemionitis levyi.*

In its authoritative revision by Tryon (1956), American *Notholaena* comprised sections *Notholaena* and *Argyrochosma,* a view reiterated by Tryon and Tryon (1982) and Tryon et al. (1990), in spite of evidence by Windham (1987) that section *Argyrochosma* deserves recognition as an independent genus. Tryon and Tryon (1982), however, did remove from section *Notholaena* to *Cheilanthes* a group of 21 species represented in this analysis by *Cheilanthes bonariensis* (species 10, previously known as *Notholaena aurea*). It nests within a clade of American *Cheilanthes* (species 7–11). The two species examined here (5, 6) that were removed from *Notholaena* to *Argyrochosma* by Windham (1987) are sister to *Pellaea* sections *Pellaea* and *Platyloma* in a clade (species 1–6) strongly supported by 10 synapomorphies and a 95% bootstrap value. These *Argyrochosma* species are deeply separated from their erstwhile congeners in *Notholaena* (represented by *N. rosei* and *N. sulphurea,* species 12 and 13), the latter two grouping instead with some species of an obviously polyphyletic *Cheilanthes.*

Cheilanthes sensu Tryon et al. (1990) is represented by 11 species in Fig. 3. Five of these (species 7–11) are united in a well-defined clade sister to the *Pellaea-Argyrochosma* clade. The other six are quite deeply separated from those five: two (species 14, 15) in a clade with *Notholaena rosei* and *N. sul-*

←

---

were used as a global outgroup (see Materials and Methods). Taxonomy and nomenclature follow Tryon et al. (1990), which presents the most recent comprehensive treatment of cheilanthoids on a worldwide basis. Parenthetical information indicates sectional taxonomy of *Pellaea* and infrageneric groups of *Cheilanthes* according to Tryon and Tryon (1982) and alternative generic taxonomies discussed in the text. Note that PAUP was unable to place *Llavea cordifolia* (species 33) as a member of a monophyletic ingroup.

phurea, two (species 18, 19) in a clade sister to that of *Hemionitis levyi* and *Pellaea boivinii,* and two (species 20, 21) in a clade with *Doryopteris* and *Trachypteris.* Based on *rbcL* sequence data, *Cheilanthes* sensu Tryon et al. (1990) is clearly polyphyletic.

Hemionitis is represented in this study by two of the seven species accepted by Tryon et al. (1990), and the genus is clearly polyphyletic under their circumscription. *Hemionitis levyi* (species 16) is sister to *Pellaea boivinii* (representing section *Holcochlaena*) in a clade with *Cheilanthes duclouxii* and *C. albofusca. Hemionitis elegans* (species 25) is very deeply separated from *H. levyi* in a clade with *Bommeria.*

Doryopteris sensu Tryon et al. (1990) is represented only by *D. pedata* var. *palmata* (species 22), which is strongly grouped by 11 synapomorphies and a 100% bootstrap value with 2 species of *Cheilanthes* (species 20, 21) that were considered species of *Doryopteris* (Tryon, 1942) prior to their transfer to *Cheilanthes* by Tryon and Tryon (1981). Those three species form a strongly supported clade with *Trachypteris* (species 23).

Bommeria is a genus of four species sensu Tryon et al. (1990), who did not accept Ranker and Haufler's (1990) transfer of *Hemionitis elegans* to *Bommeria.* Sequence data from *rbcL* place *Bommeria* as the most basal element in the cheilanthoid ferns examined here. Moreover, *B. ehrenbergiana* (species 24) is very strongly united with *H. elegans* (species 25) by 17 synapomorphies and a 100% bootstrap value in this clade sister to all other cheilanthoids.

Discussion

Llavea.—*Llavea* is a monotypic genus of the Sierra Madre Oriental of Mexico and Guatemala that is most often placed near *Cryptogramma* and *Onychium* in spite of its considerable difference from those genera (Tryon and Tryon, 1982). The contours of *Llavea*'s tuberculate spore morphology are formed by perispore, unlike the verrucate contours of *Cryptogramma* and other members of the Pteridaceae that are formed by the exospore, leading Tryon and Lugardon (1991) to conclude that *Llavea*'s spore morphology does not indicate a strong alliance with other genera of the family. *Llavea*'s spores also lack the taenitioid ridges and equatorial flange of *Onychium* (Tryon and Lugardon, 1991), which is placed far from the cheilanthoids in the global analysis of Hasebe et al. (1994; Fig. 1).

Although *Coniogramme* was used as part of the global outgroup because of its placement in a preliminary global analysis of fern phylogeny based on *rbcL* sequences (Hasebe et al., 1994; Fig. 1), *Llavea* was entered as a member of the ingroup. Based on *rbcL* sequence data, however, *Llavea* is placed far outside the cheilanthoids. The clade it forms with *Coniogramme* (Fig. 3, species 33 and 34) is sister to all the cheilanthoids (species 1–25) plus the *Adiantum-Antrophyum-Vittaria* outgroup clade (species 26–32). This analysis therefore contradicts Tryon et al. (1990) by indicating that *Llavea* is not a cheilanthoid. *Llavea*'s relationship to *Coniogramme* is strongly supported by *rbcL* data, and the perispore-based topographic relief of *Llavea*'s spores seems to differ from

that of *Coniogramme* only by degree. Nevertheless, the species representing these two genera do differ by 71 autapomorphies, suggesting that their sister relationship in this tree may be influenced by long branch length attraction (Felsenstein, 1978) that could be alleviated by adding other taxa to the analysis. To ensure that *Llavea* is not drawn to its outgroup position by some undue attraction to *Coniogramme,* the data set was reanalyzed with *Coniogramme* excluded. Four equally most parsimonious trees topologically identical to the four in the present analysis were found except that *Llavea* was placed by itself (without *Coniogramme*) between *Vittaria lineata* and *Platyzoma microphyllum* (species 32 and 35), still indicating that *Llavea* is not a cheilanthoid.

Pellaea.—Tryon et al. (1990) regarded *Pellaea* as a genus comprising four sections and about 35 species. Tryon and Tryon (1982) noted that these four sections had previously been treated as genera by various authors, but they did not consider them sufficiently distinct to merit generic rank. Section *Pellaea* sensu Tryon et al. (1990) includes the taxa revised by Tryon (1957) plus *P. bridgesii,* and thus comprises 16 American species plus South African *P. rufa. Pellaea andromedifolia, P. pringlei,* and *P. cordifolia* (Fig. 3, species 1, 2, 4) represent this section in the present study. Section *Ormopteris* contains five to seven species in South America, none yet available for *rbcL* analysis. Section *Holcochlaena* contains about 10 species from Africa and Madagascar, with *P. boivinii* (Fig. 3, species 17) included in this study. Section *Platyloma* contains three species occurring from New Zealand and Australia to New Guinea and India, with *P. rotundifolia* (Fig. 3, species 3) included in this study. A new species was added to this section by Brownsey and Lovis (1990).

Based on *rbcL* nucleotide sequences, *Pellaea* sensu Tryon et al. (1990) is polyphyletic. The alternative positions of *P. pringlei* and *P. rotundifolia,* responsible for the two equally most parsimonious ingroup topologies (Fig. 2) were discussed above. Both species have long autapomorphic branches relative to the number of synapomorphies supporting their respective clades, suggesting that their ambivalent positions may be stabilized by the addition of more taxa. The nesting of section *Platyloma*'s *P. rotundifolia* (Fig. 3, species 3) within section *Pellaea* (species 1, 2, 4) was unexpected, given their present geographic separation in New Zealand and vicinity versus America. Moreover, the consistently echinate spores of section *Platyloma* differ so strongly in surface morphology from the low rugate or cristate spores of section *Pellaea* that Tryon and Tryon (1973) regarded as convergent the similarities between leaf and soral structures of sections *Platyloma* and *Pellaea.* Tryon and Lugardon (1991) also thought that section *Platyloma* may represent a lineage distinct from the other sections of *Pellaea.* Spore morphology of section *Holcochlaena* appears to be less diverged from that of section *Pellaea* than is that of section *Platyloma* (Tryon and Lugardon, 1991). Nevertheless, section *Holcochlaena* as represented by *P. boivinii* (Fig. 3, species 17) is unexpectedly and very deeply separated from its nominal congeners, suggesting the appropriateness of generic segregation if other members of its section confirm this placement. As

discussed further under *Hemionitis* below, it is essential to add other species of section *Holcochlaena* to the *rbcL* analysis, both to avoid potentially incorrect associations influenced by the long autapomorphic branch length (Felsenstein, 1978) of *P. boivinii,* and to determine whether this species adequately represents its section (Lecointre et al., 1993).

Notholaena.—Tryon (1956) revised the American species of *Notholaena,* accepting 58 species, 7 of which were divided into 17 varieties. Tryon and Tryon (1982) removed all Old World species of *Notholaena* to *Cheilanthes,* thereby accepting in *Notholaena* only the species in the 1956 revision, except that they also transferred species 1–21 of Tryon's (1956) revision to *Cheilanthes* and accepted as *Notholaena* two of the new species described by Tryon (1961). Thus *Notholaena* in Tryon and Tryon (1982) was left as an American genus of 39 species, which they recognized in two sections (*Argyrochosma* and *Notholaena*) primarily on the basis of differences in spore morphology. Windham (1987) recognized *Notholaena* section *Argyrochosma* as an independent genus on the basis of several characters, prominent among them being the synapomorphic base chromosome number of $x=27$, which is unique among cheilanthoids. He also noted that the characters that differentiate *Argyrochosma* from *Notholaena* indicate a close and potentially sister relationship between *Argyrochosma* and *Pellaea* section *Pellaea.* Tryon et al. (1990) did not accept generic rank for *Argyrochosma,* maintaining Tryon and Tryon's (1982) circumscription as an American genus of about 40 species.

PAUP's analysis of *rbcL* sequence data addresses several of these modifications of Tryon's (1956) treatment of *Notholaena.* Species 5, 6, 10, 12, and 13 of Fig. 3 are species of *Notholaena* sensu Tryon (1956). Their distribution among three major clades in this analysis indicates that *Notholaena* sensu Tryon (1956) is polyphyletic. The separation of *Notholaena delicatula* and *N. fendleri* (species 5 and 6) from *N. rosei* and *N. sulphurea* (species 12 and 13) at the second-deepest level of the tree very strongly supports recognition of *Notholaena* section *Argyrochosma* at generic rank, as suggested by Copeland (1947, p. 70) and Weatherby (in Morton, 1950, pp. 249–250) and effected by Windham (1987). Moreover, *Argyrochosma,* as represented by these two species, is phylogenetically sister to *Pellaea* section *Pellaea* (species 1, 2, 4), as Windham (1987) proposed. In this analysis, *Cheilanthes bonariensis* (species 10; *Notholaena aurea* sensu Tryon, 1956) is the only representative of the first 21 species of *Notholaena* sensu Tryon (1956) transferred to *Cheilanthes* by Tryon and Tryon (1982). Its position in a clade (species 7–11) exclusively containing four traditional *Cheilanthes* species suggests that the transfer of the species group it represents to *Cheilanthes* was as appropriate as the elevation of section *Argyrochosma* to generic rank.

Cheilanthes.—*Cheilanthes* has not received modern revisionary study. The most comprehensive recent taxonomic overview of the genus is that of Tryon and Tryon (1982), which focused on American species, but commented on the genus as a whole. That account distributed the American species into 10 groups plus a set of morphologically isolated species, ". . . to show the main

lines of diversity in America and to provide a framework for further studies" (Tryon and Tryon, 1982, pp. 251–255). Tryon et al. (1990, p. 242) noted that on a worldwide basis, ". . . perhaps 30 [unspecified] groups could be recognized in *Cheilanthes.*" Both the 1982 and 1990 treatments recognized that many mostly small and sometimes monotypic genera have been segregated from *Cheilanthes,* but most of those segregate genera were not accepted, on the grounds that they, ". . . represent only a portion of the diversity in the genus" (Tryon et al., 1990). This *rbcL* analysis includes a small selection of both traditional species of *Cheilanthes* and representatives of some of the segregate genera that were not accepted by Tryon and Tryon (1982) and Tryon et al. (1990).

Species 7–11 of Fig. 3 form a clade of five American *Cheilanthes* species distributed among four of the groups recognized by Tryon and Tryon (1982). This entire clade of *Cheilanthes* is sister to the clade (species 1–6) of *Pellaea* (sections *Pellaea* and *Platyloma*) plus *Argyrochosma.* The perception of Tryon and Tryon's (1982) transfer of *Notholaena aurea* (species 10) to *Cheilanthes* as *C. bonariensis* is supported by *rbcL* analysis. How well *C. bonariensis* represents its entire group of 21 species transferred from *Notholaena* will be determined by incorporating additional species from this group into an expanded *rbcL* analysis. Based on the present small sample of species, the ten groups of American *Cheilanthes* in Tryon and Tryon (1982) may not be phylogenetically natural, given that *C. lanosa* and *C. bonariensis* (both from group 7) are in the most deeply separated subdivisions of this clade. *Cheilanthes intramarginalis* (species 11) is sometimes placed in the segregate genus *Mildella* discussed below.

Other species of *Cheilanthes* (Fig. 3, species 14, 15, 18–21) are very deeply separated from the core of American species represented by species 7–11, indicating that *Cheilanthes* sensu Tryon et al. (1990) is polyphyletic. Mexican *C. rigida* and *C. aurea* (species 14, 15) form a strongly supported clade (eight synapomorphies, 97% bootstrap value) with traditional *Notholaena* (species 12, 13). *Cheilanthes aurea* is placed in *Cheilanthes* group 4 by Tryon and Tryon (1982). Species of this group share with *Notholaena* a whitish or yellowish waxy farina on the underside of the lamina, as do the far removed species of *Argyrochosma* (species 5, 6) and *Cheilanthes albofusca* and *C. duclouxii* (species 18, 19) discussed below. Mere presence or absence of waxy farina is evidently homoplastic, although particular chemical constituents of this farina may be found to correlate with the phylogenetic arrangement of taxa beginning to emerge from these sequence data. This should be investigated after more taxa are subjected to the *rbcL* analysis. *Cheilanthes rigida* (species 14) was placed by Tryon and Tryon (1982) in *Cheilanthes* group 11, a set of morphologically isolated species they considered incertae sedis. Its long autapomorphic branch length suggests that its close relatives have not yet been included in the *rbcL* analysis. It is sometimes placed in the segregate genus *Cheiloplecton* discussed below.

Cheilanthes albofusca and *C. duclouxii* (species 18, 19) are Asiatic species often placed in segregate genera *Sinopteris* and *Aleuritopteris,* respectively

(discussed below). Their segregate status is strongly supported by their basal separation from the core of American *Cheilanthes* represented by species 7–11. Although strongly linked to each other by six synapomorphies, their sister relationship to *Hemionitis levyi* and *Pellaea boivinii* (species 16, 17) is more tenuous.

Hawaiian *Cheilanthes decora* and pantropical *C. concolor* (species 20, 21) were regarded as species of *Doryopteris* (Tryon, 1942) until Tryon and Tryon (1981) removed them and about five other species of Africa-Madagascar to an independent group in *Cheilanthes* (group 3 of Tryon and Tryon, 1982). Based on *rbcL* sequence data, they form a very strongly supported clade (11 synapomorphies, 100% bootstrap value) with Mexican *Doryopteris pedata* var. *palmata*. Unlike Tryon and Tryon's (1982) transfer of *Notholaena aurea* to *Cheilanthes bonariensis*, this transfer of erstwhile *Doryopteris* species to *Cheilanthes* is not supported by molecular data.

GENERA SEGREGATED FROM *CHEILANTHES*.—Mickel and Beitel (1988, p. 100) noted that, "There are several natural species groupings within *Cheilanthes*, but at what level they should be recognized is still one of the major questions in fern taxonomy." Tryon et al. (1990, p. 242) listed *Mildella*, *Cheiloplecton*, *Sinopteris*, and *Aleuritopteris* among the small genera segregated from *Cheilanthes* that they did not recognize. Representatives (Fig. 3, species 11, 14, 18, 19) of these segregate genera are included in the present analysis. *Cheilanthes intramarginalis* (species 11) is sometimes segregated as a species of *Mildella* (Hall and Lellinger, 1967). It shares 8–16 synapomorphies with other members of the clade of American *Cheilanthes* (species 7–11) discussed above, but it is set apart by 18 autapomorphies in this analysis. The addition of other *Mildella* taxa to the *rbcL* analysis will likely further clarify the relationships of this species, but the present analysis indicates that *Cheilanthes* would be paraphyletic if *Mildella* were recognized as an independent genus. *Cheilanthes rigida* (species 14) is sometimes placed in the segregate genus *Cheiloplecton* (Smith, 1981; Mickel and Beitel, 1988; our specimen would be regarded as *Cheiloplecton rigidum* (Sw.) Fée var. *lanceolatum* C.C. Hall ex Mickel & Beitel), but was considered incertae sedis by Tryon and Tryon (1982) who placed it among the morphologically isolated species of their group 11. It is clearly phylogenetically removed from the core of American *Cheilanthes* in which *Mildella* is found, forming a clade (species 12–15) with *Notholaena* and farinose *C. aurea*. Based on this evidence, *C. rigida* should be segregated from *Cheilanthes* at the generic level. However, the four species in this clade are each characterized by numerous autapomorphies, suggesting that their respective close affinites are yet to be elucidated. Whether *C. rigida* should be considered congeneric with one or more of the species in its clade or generically independent remains to be seen. Chinese *C. albofusca* and *C. duclouxii* (species 18, 19), representing segregate genera *Sinopteris* and *Aleuritopteris* respectively (Christensen and Ching, 1933; Ching, 1941), are even more deeply removed from the clade of American *Cheilanthes* than is *C. rigida*, suggesting that segregate status is warranted. Inclusion of additional *Aleuritopteris* spe-

cies will help to determine whether these taxa should be combined into a single segregate genus.

Hemionitis.—Tryon et al. (1990) considered *Hemionitis* a tropical American genus of seven species. Based on data from morphology, flavonoids, and allozymes, Ranker (1990) accepted five of these species, but transferred *H. elegans* to *Bommeria* (Ranker, 1990; Ranker and Haufler, 1990) and accepted Mickel's (1987) transfer of *H. subcordata* to *Cheilanthes*. The diverse surface and wall structure of *Hemionitis* spores led Tryon and Lugardon (1991) to suggest that the genus sensu Tryon et al. (1990) represents a polymorphic group. Two species sensu Tryon et al. (1990; Fig. 3, species 16 and 25) were included in this analysis, and the results clearly indicate that *Hemionitis* in this sense is polyphyletic. *Hemionitis levyi* (species 16), in a clade with *Pellaea boivinii* (species 17), has 31 autapomorphies, suggesting that its association with *P. boivinii* may be influenced by branch length attraction (Felsenstein, 1978). Furthermore, because this analysis shows that neither *P. boivinii* nor *H. levyi* group with their nominal congeners sensu Tryon et al., additional species of both *Hemionitis* and *Pellaea* section *Holcochlaena* must be included in an expanded *rbcL* analysis in view of the importance to clade robustness of sampling several species per presumed monophyletic group (Lecointre et al., 1993). It seems clear, however, that *H. elegans* (species 25) should be removed from *Hemionitis* to *Bommeria* as was done by Ranker and Haufler (1990) on the basis of morphology, isozymes, and flavonoids.

Doryopteris.—*Doryopteris* sensu Tryon et al. (1990) is distributed from tropical America to the Old World, with its 25 species assigned to distinct evolutionary lines as sections *Lytoneuron* and *Doryopteris* (Tryon and Tryon, 1982). *Doryopteris concolor* and *D. decora* of Tryon (1942) were transferred to *Cheilanthes* by Tryon and Tryon (1981). In this revised sense, only *D. pedata* var. *palmata* (Fig. 3, species 22) from section *Doryopteris* is represented in this analysis, although *D. concolor* and *D. decora* are represented as species of *Cheilanthes* sensu Tryon et. al (1990). As discussed above under *Cheilanthes,* the clade (Fig. 3, species 20–22) formed by these two species and *D. pedata* is very strongly supported by 11 synapomorphies and a 100% bootstrap value and is deeply separated from the clade of American *Cheilanthes* (species 7–11). Based on *rbcL* sequence data, *C. decora* and *C. concolor* must be returned to *Doryopteris*. Based on morphological differences, Tryon and Tryon (1982) concluded that the paleotropical species of section *Doryopteris* may constitute a convergent element derived from a different source than the neotropical ones. Additional species of section *Doryopteris* and species of section *Lytoneuron* need to be added to the *rbcL* analysis to test the monophyly of the genus.

Trachypteris.—*Trachypteris* is a genus of three species, two South American and one in Madagascar (Tryon et al., 1990), an unusual distribution similar to that of *Doryopteris* section *Doryopteris* (Tryon and Tryon, 1982). Although the pedate form of *Trachypteris* sporophylls is reminiscent of the pedate lamina

architecture in *Doryopteris,* Tryon and Tryon (1982) regarded *Trachypteris* as a specialized genus of unclear relationships within the cheilanthoid ferns. Tryon and Tryon (1973) observed that the strongly cristate sporoderm of *Trachypteris induta* (as *Saffordia induta*) is consistent with its position next to *Doryopteris* in Christensen's (1938) treatment, but concluded that the relationship of *Trachypteris* to other cheilanthoids is not clear. Tryon and Lugardon (1991) felt that spore morphology supported treating these three species as congeneric, but reiterated the view that the close generic affinities of *Trachypteris* within the cheilanthoids are unclear. Based on *rbcL* sequence data, *Trachypteris* (Fig. 3, species 23) is strongly supported as sister to the *Doryopteris* clade (species 20–22), linked to *Doryopteris* (including the species inappropriately transferred to *Cheilanthes* group 3) by nine synapomorphies with a 91% bootstrap confidence value. This relationship appears to be consistent with the spore morphology of these two genera as presented by Tryon and Lugardon (1991), except for the exceptional spore morphology of the probably misplaced species *Doryopteris papuana.* The long autapomorphic branch length of *Trachypteris* suggests that relationships between it and *Doryopteris* may become better resolved as more taxa in this assemblage become available for molecular analysis.

Bommeria.—Tryon et al. (1990) followed the revision by Haufler (1979) in accepting four species of *Bommeria,* but Ranker (1990) and Ranker and Haufler (1990) transferred *Hemionitis elegans* to *Bommeria,* as noted in the discussion of *Hemionitis* above. The sister relationship of *B. ehrenbergiana* and *H. elegans* (Fig. 3, species 24, 25) is more strongly supported (17 synapomorphies and a 98% bootstrap value) than is any other relationship in this study. In addition, this analysis establishes *Bommeria* as the most basal clade in the cheilanthoids as circumscribed by Tryon et al. (1990), given that *Llavea* is not a cheilanthoid, as discussed above.

Reliability of Inferences based on *rbcL.*—Phylogenetic relationships reconstructed from *rbcL* sequence data in this study are concordant with taxonomic realignments based on broad suites of characters in recent studies of small groups of cheilanthoid species. Examples discussed above are the segregation of *Argyrochosma* from *Notholaena* and the transfer of *Hemionitis elegans* to *Bommeria.* This concordance suggests that *rbcL* will also provide long-sought better insights into the phylogeny and generic circumscriptions of troublesome larger cheilanthoid groups less amenable to studies employing a broad suite of characters. The larger traditional taxa discussed at the beginning of this paper were established through a taxonomic methodology whereby well-informed, experienced taxonomists subjectively weight and interpret character states whose genetic basis and potential homoplasy are undetermined. When this same subjective methodology is used to revise traditional circumscriptions, the results may or may not be concordant with those based on cladistically analyzed molecular data. For example, Tryon and Tryon (1982), perhaps the most experienced students of cheilanthoid ferns, transferred to *Cheilanthes* the first 21 species formerly assigned to *Notholaena* (Tryon, 1956). This trans-

fer was apparently based on their experienced assessment that those species are not closely related to the predominantly farinose [*Notholaena*] species of America (Tryon and Tryon, 1981). Based on the single species *Cheilanthes bonariensis* (formerly *Notholaena aurea*) used here to represent that group, cladistic analysis of *rbcL* sequences supports their new hypothesis of the relationships of those 21 species. On the other hand, their transfer of the group of *Doryopteris concolor* sensu Tryon (1942) to *Cheilanthes* group 3 (Tryon and Tryon, 1981, 1982) is strongly rejected by *rbcL* sequence data, which instead support Tryon's earlier (1942) generic placement.

The genus by genus discussion above indicates that many additional cheilanthoid taxa should be incorporated into the *rbcL* analysis. Sequences from *Adiantopsis, Paraceterach,* and *Cryptogramma* were not available for inclusion in this study, but will be included in an expanded analysis. Long autapomorphic branch lengths of some ingroup species in Fig. 3 indicate areas where additional species of genera already in the study should be added, both to establish the closer affinities of these highly autapomorphic species and to avoid possible artifactual relationships resulting from long branch attractions (Felsenstein, 1978). Increased species representation in some cases will also help to avoid potential distortions inherent in phylogenetic inferences based on inadequate species sampling (Lecointre et al., 1993). The present study offers insights into where additional species representation will be most useful.

Although cladistic analysis of *rbcL* sequences promises long-sought insights into cheilanthoid phylogeny, recent studies of flowering plants have found instances in which hybridization, introgression, and lineage sorting may lead to erroneous reconstructions of species lineages when reconstructions are based on the maternally inherited chloroplast genome (e.g., Rieseberg and Soltis, 1991; Doyle, 1992). This raises concerns for cheilanthoids and other homosporous ferns because their chloroplast genomes (including *rbcL*) are maternally inherited (Gastony and Yatskievych, 1992) and because their high incidence of allopolyploid hybrids and high basic chromosome numbers suggest that modern ferns may be paleopolyploids in whose ancestry reticulate evolution may have been important (Gastony, 1991). In that case, chloroplast DNA gene trees and species trees based on biparentally inherited nucleus-encoded characters (e.g., morphology) could be discordant. It would be inappropriate to test concordance of chloroplast and nuclear data sets by employing nucleus-encoded *morphological* characters because our current problems in interpreting cheilanthoid phylogeny have been attributed to habitat-related homoplastic morphology, as discussed above. To circumvent potential morphological homoplasy while addressing concerns about concordance of chloroplast- and nucleus-derived phylogenies in ferns, we have begun generating molecular (sequence) data from biparentally inherited, nucleus-encoded, ribosomal DNA genes (Gastony, 1994). Direct comparison of cheilanthoid phylogenies reconstructed from molecular data in these two genomes will help to establish whether potential inconsistencies between *rbcL* relationships and traditional relationships are attributable to morphological homoplasy associated with ad-

aptation to xeric habitats as some have suggested or to discordant phylogenetic histories experienced by maternally and biparentally inherited genomes.

Acknowledgments

We appreciate Collen K. Dixon's work in generating double-stranded sequence for some members of the ingroup during early stages of this study and the assistance of George Yatskievych in obtaining many of the ingroup plants. We thank Jianwei Li, Tim Flynn, Adrian Hegeman, Ramón Riba, Kelly Sullivan, Sigrid Liede, and Paulo Windisch for supplying the plant material indicated in Table 1. Mitsuyasu Hasebe and Edmund Crane generously provided the sequences of *Doryopteris concolor* and the outgroup taxa. GJG is most grateful to Drs. Rolla and Alice Tryon, both for the graduate training that inspires this research and for their monumental contributions to cheilanthoid systematics on which this work is based. This study was supported by National Science Foundation grants BSR-8817981 and DEB-9307068 to GJG.

Literature Cited

Archie, J. W. 1989. Homoplasy excess ratios: New indices for measuring levels of homoplasy in phylogenetic systematics and a critique of the consistency index. Syst. Zool. 38:253–269.

Brownsey, P. J., and J. D. Lovis. 1990. *Pellaea calidirupium*—a new fern species from New Zealand. New Zealand J. Bot. 28:197–205.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y.-L. Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedrén, B. S. Gaut, R. K. Jansen, K.-J. Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q.-Y. Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, Jr., S. W. Graham, S. C. H. Barrett, S. Dayanandan, and V. A. Albert. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbcL*. Ann. Missouri Bot. Gard. 80:528–580.

Ching, R. C. 1941. The studies of Chinese ferns—XXXI. Hong Kong Naturalist 10:194–204.

Christensen, C. 1938. Filicinae. Pp. 522–550 *in* F. Verdoorn, ed. *Manual of pteridology*. Martinus Nijhoff, The Hague.

Christensen, C., and R. C. Ching. 1933. *Sinopteris*, a new fern genus in China. Bull. Fan Mem. Inst. Biol. 4:355–364.

Copeland, E. B. 1947. *Genera filicum*. Chronica Botanica, Waltham, MA.

Doyle, J. J. 1992. Gene trees and species trees: Molecular systematics as one-character taxonomy. Syst. Bot. 17:144–163.

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27:401–410.

———. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Gastony, G. J. 1991. Gene silencing in a polyploid homosporous fern: Paleopolyploidy revisited. Proc. Natl. Acad. Sci. U.S.A. 88:1602–1605.

———. 1994. Phylogenetic relationships among genera of cheilanthoid ferns (Pteridacae: Cheilanthoideae) inferred from gene sequence data. Amer. J. Bot. 81(6, suppl.):120. [Abstract]

Gastony, G. J., and G. Yatskievych. 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. Amer. J. Bot. 79:716–722.

Hall, C. C., and D. B. Lellinger. 1967. A revision of the fern genus *Mildella*. Amer. Fern J. 57: 113–134.

Hasebe, M., P. G. Wolf, W. G. Hauk, J. R. Manhart, C. H. Haufler, R. Sano, G. J. Gastony, N. Crane, K. M. Pryer, N. Murakami, J. Yokoyama, and M. Ito. 1994. A global analysis of

fern phylogeny based on *rbcL* nucleotide sequences. Amer. J. Bot. 81(6 suppl.):120–121. [Abstract]

HASEBE, M., P. G. WOLF, K. M. PRYER, K. UEDA, M. ITO, R. SANO, G. J. GASTONY, J. YOKOYAMA, J. R. MANHART, N. MURAKAMI, E. H. CRANE, C. H. HAUFLER, and W. D. HAUK. 1995. Fern phylogeny based on *rbcL* nucleotide sequences. Amer. Fern J. 85:134–181.

HAUFLER, C. H. 1979. A biosystematic revision of *Bommeria*. J. Arnold Arbor. 60:445–476.

———. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. 10:92–104.

HILLIS, D. M., A. LARSON, S. K. DAVIS, and E. A. ZIMMER. 1990. Nucleic acids III: Sequencing. Pp. 318–370 *in* D. M. Hillis and C. Moritz, eds. *Molecular systematics*. Sinauer Associates, Sunderland, MA.

KNOBLOCH, I. W. 1976. Morphological characters in *Cheilanthes* together with a key to North and Central American species. Flora 165:507–522.

LECOINTRE, G., H. PHILIPPE, H. L. V. LÊ, and H. L. GUYADER. 1993. Species sampling has a major impact on phylogenetic inference. Molec. Phylog. Evol. 2:205–224.

LELLINGER, D. B. 1968. A note on *Aspidotis*. Amer. Fern J. 58:140–141.

———. 1985. *A field manual of the ferns and fern-allies of the United States and Canada*. Smithsonian Institution Press, Washington, D.C.

———. 1989. The ferns and fern-allies of Costa Rica, Panama, and the Chocó (Part 1: Psilotaceae through Dicksoniaceae). Pteridologia 2A:1–364.

MADDISON, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Syst. Zool. 40:315–328.

MICKEL, J. T. 1974. A redefinition of the genus *Hemionitis*. Amer. Fern J. 64:3–12.

———. 1979. The fern genus *Cheilanthes* in continental United States. Phytologia 41: 431–437.

———. 1987. A new fern from western Mexico and its bearing on the taxonomy of the cheilanthoid ferns. Amer. Fern J. 77:109–114.

MICKEL, J. T., and J. M. BEITEL. 1988. Pteridophyte flora of Oaxaca, Mexico. Mem. New York Bot. Gard. 46:1–568.

MORTON, C. V. 1950. Notes on the ferns of the eastern United States (concluded). Amer. Fern J. 40:241–252.

RANKER, T. A. 1990. Phylogenetic systematics of neotropical *Hemionitis* and *Bommeria* (Adiantaceae) based on morphology, allozymes, and flavonoids. Syst. Bot. 15: 442–453.

RANKER, T. A., and C. H. HAUFLER. 1990. A new combination in *Bommeria* (Adiantaceae). Amer. Fern J. 80:1–3.

RIESEBERG, L. H., and D. E. SOLTIS. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends Pl. 5:65–84.

SAGHAI-MAROOF, M. A., K. M. SOLIMAN, R. A. JORGENSEN, and R. W. ALLARD. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. U.S.A. 81:8014–8018.

SAIKI, Y. 1984. Notes on ferns (2). Asiatic species of the *Aleuritopteris farinosa* complex. J. Phytogeogr. Taxon. 32:81–98.

SAMBROOK , J. E., F. FRITSCH, and T. MANIATIS. 1989. *Molecular cloning, a laboratory manual*, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SMITH, A. R. 1975. The California species of *Aspidotis*. Madroño 23:15–24.

———. 1981. *Pteridophytes*. Part 2 *in* D. E. Breedlove, ed. *Flora of Chiapas*. California Academy of Sciences, San Francisco, CA.

STOLZE, R. G. 1981. Ferns and fern allies of Guatemala, part 2. Fieldiana Bot. (n.s.) 6:1–522.

SWOFFORD, D. L. 1993. *PAUP: Phylogenetic analysis using parsimony, version 3.1. User's manual*. Illinois Natural History Survey, Champaign, IL.

TRYON, A. F., and B. LUGARDON. 1991. *Spores of the Pteridophyta*. Springer-Verlag, New York.

TRYON, R. M., JR. 1942. A revision of the genus *Doryopteris*. Contr. Gray Herb. 143:1–80.

———. 1956. A revision of the American species of *Notholaena*. Contr. Gray Herb. 179:1–106.

TRYON, R. M., JR., and A. F. TRYON. 1973. Geography, spores, and evolutionary relations in the cheilanthoid ferns. Pp. 145–153 *in* A. C. Jermy, J. A. Crabbe, and B. A. Thomas, eds. The phylogeny and classification of the ferns. Bot. J. Linn. Soc. 67 (suppl. 1):i–xiv, 1–284.

———. 1981. Taxonomic and nomenclatural notes on ferns. Rhodora 83: 133–137.

———. 1982. *Ferns and allied plants with special reference to tropical America.* New York: Springer-Verlag.

TRYON, R. M., JR., A. F. TRYON, and K. U. KRAMER. 1990. Pteridaceae. Pp. 230–256 *in* K. Kubitzki, ed. 1990. *The families and genera of vascular plants. Vol. 1. Pteridophytes and gymnosperms.* Vol. eds. K. U. Kramer and P. S. Green. Springer-Verlag, Berlin.

WINDHAM, M. D. 1987. *Argyrochosma,* a new genus of cheilanthoid ferns. Amer. Fern J. 77:37–41.

YATSKIEVYCH, G., D. B. STEIN, and G. J. GASTONY. 1988. Chloroplast DNA evolution and systematics of *Phanerophlebia* (Dryopteridaceae) and related fern genera. Proc. Natl. Acad. Sci. U.S.A. 85:2589–2593.

ZURAWSKI, G., and M. T. CLEGG. 1993. *RbcL* sequence data and phylogenetic reconstructions in seed plants: Forward. Ann. Missouri Bot. Gard. 80:523–525.